Homology between IRE-BP, a regulatory RNA-binding protein, aconitase, and isopropylmalate isomerase

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ABSTRACT

Iron-responsive elements (IREs) are regulatory RNA elements which serve as specific binding sites for the IRE-binding protein (IRE-BP). Interaction between IREs and IRE-BP induces repression of ferritin mRNA translation and transferrin receptor mRNA stabilization. We describe the identification of extensive amino acid sequence homology between IRE-BP and two known isomerases, aconitase and isopropylmalate (IPM) isomerase. We discuss the implications of this observation with regard to structure/function relationships of IRE-BP. The structural conservation between a regulatory RNA-binding protein and two enzymes involved in intermediary metabolism provides a surprising example of the functional flexibility in biological structures.

INTRODUCTION

Similarities among protein sequences can help to identify the function of a newly characterized gene product and can provide structural information about homologous domains of different proteins. We have noticed extensive amino acid sequence homology between the iron-responsive element binding protein [IRE-binding protein, IRE-BP] and the Krebs' cycle enzyme aconitase whose crystal structure has been recently solved (1,2). The homology provides considerable structural information applicable to IRE-BP and should advance our understanding of IRE-BP function as a cytoplasmic RNA-binding protein and as a post-transcriptional regulator.

IRE-BP binds to iron-responsive elements (IREs) which were first identified in the 5′ UTR of ferritin mRNA and in the 3′ UTR of transferrin receptor (TfR) mRNA (3, 4, 5). Binding of IRE-BP to IREs is regulated by the iron status of the cell. Iron starvation activates binding and thus represses ferritin mRNA translation and stabilizes TfR transcripts *in vivo*. The effect of changes in iron availability *in vivo* can be mimicked by alterations in the redox environment of IRE-BP *in vitro* (6, 7). This observation has led to the suggestion that IRE-BP activity is regulated post-translationally by the reversible oxidation-reduction of cysteinyl sulfhydryl groups important for the interaction of IRE-BP and IREs.

METHODS

The amino acid sequences were aligned according to the procedures of Argos (8) which are sensitive to distant relationships as both residue physical characteristics and the Dayhoff residue substitution scoring matrix are utilized to evaluate the matches. Once a consensus sequence motif (see Figure 1 caption) had been delineated from the alignment of IRE-BP and aconitase, the computer programm SCRUTINEER (9) was used to search a database of protein sequences for other possible family members. SCRUTINEER allows for flexible pattern definitions and searches quickly the large databases. The database searched was SWISSPROT, version 15.0, containing over 18000 primary structures (10). The three sequences in Figure 1 were then aligned by first matching the closest pair (aconitase and IRE-BP) and then adding IPM through its close alignment with aconitase.

RESULTS AND DISCUSSION

Recently, Rouault et al. (11) reported the cloning of the cDNA for human IRE-BP; the authors discussed the presence of the amino acid sequence CXXC, which occurs in several proteins containing iron-sulfur (Fe-S) clusters. We noticed that a classical Fe-S protein, aconitase, which exists in a mitochondrial and a cytoplasmic form and catalyzes the isomerization between citrate and isocitrate, shares several characteristics with IRE-BP. The activity of both proteins can be modulated by treatment with oxidizing or reducing agents, both have a molecular weight of 85−90 kD, and the genes for both IRE-BP and the cytoplasmic form of aconitase are located on human chromosome 9 (11, 12, 13). The gene encoding mitochondrial aconitase is located on human chromosome 22. We compared the deduced amino acid sequence of human IRE-BP with that of mitochondrial aconitase from porcine heart (14); the cDNA or amino acid sequence of cytoplasmic aconitase is not known. The two proteins could be aligned along their entire lengths (Figure 1) using a sensitive comparison technique (8, 15). Overall, 214 out of 706 [30.3%] amino acids are identical, and 57.2% are conserved. The statistical significance of the alignment was 8.4 standard deviations (s) according to the strict criteria of Rechid et al. (15) who consider sequence biases arising from structural features generally shared by proteins. A systematic search of the entire Swissprot amino acid sequence database (version 15.0) revealed

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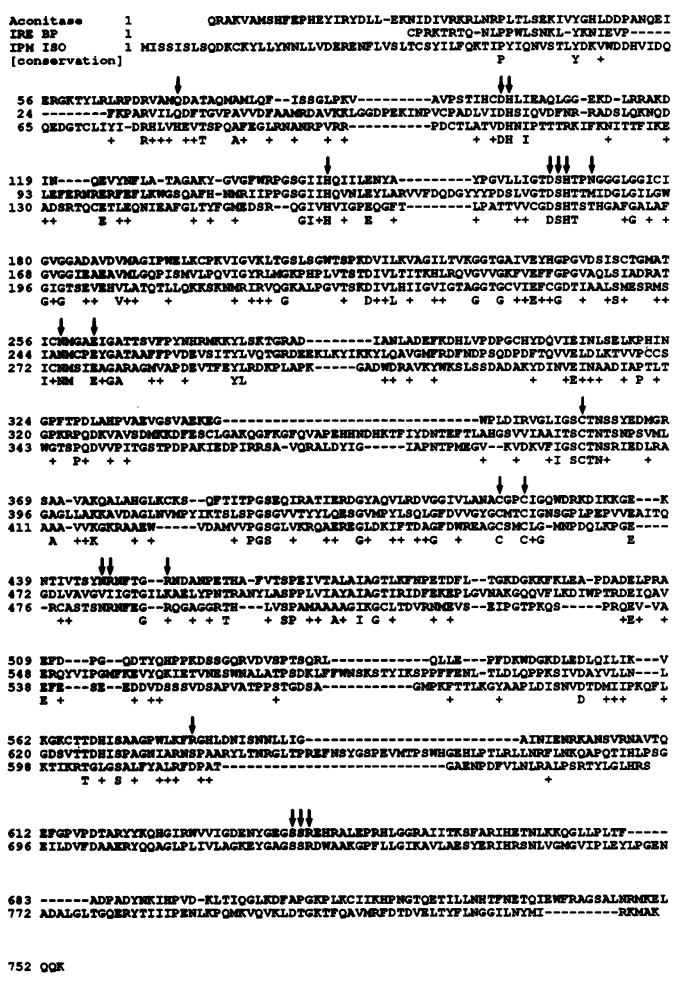

Figure 1: Alignment of the amino acid sequence for IRE-BP, porcine heart aconitase and IPM isomerase from *Mucor circinelloides*. The sequences were aligned according to the method of Argos (1987). The entire SWISSPROT protein sequence database (10) was searched for sequences containing the pattern SCTN, 50–70 residues of any type, and then CXXC where x is any amino acid. The computer program SCRUTINEER (9) was utilized for this purpose. The pattern was chosen as the aconitase tertiary structure showed the three cysteines to be iron ligands (1). If residues are identically conserved in all three sequences, the single letter code is repeated in the [conservation] row. A '+' is used to indicate conservation in all three sequences according to the following groups: [P, G], [S, T], [K, R], [Q, D, E, N], and [A, I, V, L, M, C, W, Y, H, F]. Arrows indicate the 20 likely active site residues in the tertiary structure of porcine heart aconitase as defined by Robbins and Stout (1). Other sequences which can be easily aligned with those shown here but not included are mitochondrial aconitase from *Saccharomyces cerevisiae* (20) and *leuC* from *Salmonella typhimurium* (21).

that the primary structure of isopropylmalate (IPM) isomerase from *Mucor circinelloides* (16) was significantly related to that of aconitase [23.2% identity and 51.2% conservation in 578 matched residues; $\sigma=4.7$] and that of IRE-BP [22.5% identity and 47.2% conservation in 591 aligned residues; $\sigma=3.4$] (Figure 1). IPM isomerase catalyzes the second step in the leucine biosynthetic pathway of several microorganisms (17, 18), and thus, similar to aconitase mediates an isomerization reaction, whereas no enzymatic properties have been suggested for IRE-BP; yet, aconitase and IRE-BP are more closely related than the two isomerases.

Aconitase occurs in an active and in an inactive form. The inactive form contains an [3Fe-4S] cluster which can be activated *in vitro* by introduction of a fourth Fe^{2+} under reducing conditions. The fourth Fe allows the [4Fe-4S] cluster to form and is directly involved in substrate coordination. The conversion of the [3Fe-4S] cluster into an [4Fe-4S] cluster is isomorphous (reviewed by Beinert and Kennedy (19)) and the coordination sites of the Fe ions have been determined. The [3Fe-4S] cluster is liganded by cysteines 358, 421, and 424. The fourth ligand to the [4Fe-4S] cluster is water or hydroxyl (1). The IRE-BP displays strong conservation of these three cysteines at positions 385, 451, and 454 (C_{451} and C_{454} correspond to the CXXC motif discussed by Rouault et al. (11). In addition to the three cysteine residues, the crystal structure of aconitase suggested 17 amino acids extending from Q_{72} to R_{644} as active site residues [indicated by arrows in Fig. 1]. The amino acid sequence of IRE-BP is identical in 12 of these 17 aconitase positions. Based on the extensive overall conservation of 57.2% and the 75% [15/20] identity of functionally critical amino acids, we suggest that IRE-BP is an Fe-S protein whose tertiary fold closely resembles that of aconitase.

As a direct consequence of this structural resemblance, mutagenesis experiments to explore structure/function relationships of IRE-BP can be initiated. It is tempting to speculate that a redox-sensitive interconversion between [3Fe-4S] and [4Fe-4S] clusters of IRE-BP constitute, at least in part, the intracellular mechanism which senses changes in the cellular iron state. The similarity amongst IRE-BP and the two isomerases raises the question whether IRE-BP has enzymatic activity and what would be the physiological substrate(s). Furthermore, a presently unrecognized RNA-binding function of aconitase and IPM isomerase has to be considered. We note that it may even be possible that cytoplasmic aconitase and IRE-BP are the same protein. Future experiments may help to identify other members of this unusual 'family', especially with the present description of the important sequence motifs.

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